AR TA Assays Using Human Cells

Reference	Wang and Fondell (2001)	Wang and Fondell (2001)
Characteristics of Cell Line		
Name of cell line	HeLa (E19)	HeLa (E19)
Cell source	Human cervical tumor	Human cervical tumor
Transfection of Cells with Plasmids		
Stable or transient tranfection	Stable AR/Transient reporter	Stable AR/Transient reporter
AR expression vector	pTetCMV-F0(S)-AR	pTetCMV-F0(S)-AR
AR source	human	human
Reporter vector	MMTV-Luc	ARE2-DS-Luc
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	none	none
Endpoint measured for cell toxicity	n.a.	n.a.
Preparation of Cells for Assay		
Transient		
Pregrowth of cells before transient transfection	One day prior to transfection with reporter vector	One day prior to transfection with reporter vector
Time from transient transfection to treatment of cells	3 hours	3 hours
Stable		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Test substance solvent	n.p.	n.p.
No. of replicates	n.p.	n.p.
No. of times assay repeated	3	3
Test substance incubation time	48 hours	48 hours
Agonism assay		
Reference ligand	Testosterone	Testosterone
Final concentration of reference ligand	100 nM	100 nM
Antagonism assay		
Reference ligand	Testosterone	Testosterone
Final concentration of reference ligand	n.p.	n.p.

Abbreviations: n.a. = not applicable;
n.p. = not provided

AR TA Assays Using Human Cells

Reference	Wang and Fondell (2001)	Gaido et al. (2000)
Characteristics of Cell Line		
Name of cell line	HeLa (E19)	HepG2
Cell source	Human cervical tumor	Human hepatoma
Transfection of Cells with Plasmids		
Stable or transient tranfection	Stable AR/Transient reporter	Transient AR/Transient reporter
AR expression vector	pTetCMV-F0(S)-AR	n.p.
AR source	human	human
Reporter vector	PB(-285/+32)-Luc	MMTV-Luc
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	none	pCMVβ-gal
Endpoint measured for cell toxicity	n.a.	β-galactosidase activity
Preparation of Cells for Assay		
Transient		
Pregrowth of cells before transient transfection	One day prior to transfection with reporter vector	overnight
Time from transient transfection to treatment of cells	3 hours	n.p.
Stable		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Test substance solvent	n.p.	n.p.
No. of replicates	n.p.	3
No. of times assay repeated	3	At least 3
Test substance incubation time	48 hours	24 hours
Agonism assay		
Reference ligand	Testosterone	n.a.
Final concentration of reference ligand	100 nM	n.a.
Antagonism assay		
Reference ligand	Testosterone	5α-Dihydrotestosterone
Final concentration of reference ligand	n.p.	n.p.

Abbreviations: n.a. = not applicable;
n.p. = not provided

AR TA Assays Using Human Cells

Reference	Maness et al. (1998)	Tamura et al. (2001)
Characteristics of Cell Line		
Name of cell line	HepG2	HepG2
Cell source	Human hepatoma	Human hepatoma
Transfection of Cells with Plasmids		
Stable or transient tranfection	Transient AR/Transient reporter	Transient AR/Transient reporter
AR expression vector	pRSAR	pRSAR
AR source	human	human
Reporter vector	MMTV-Luc	MMTV-Luc
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	pCMVβ-gal	pCMVβ-gal
Endpoint measured for cell toxicity	β-galactosidase activity	β-galactosidase activity
Preparation of Cells for Assay		
Transient		
Pregrowth of cells before transient transfection	overnight	n.p.
Time from transient transfection to treatment of cells	3 hours	n.p.
Stable		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Test substance solvent	DMSO	DMSO
No. of replicates	3	3
No. of times assay repeated	3 to 9	3
Test substance incubation time	24 hours	24 hours
Agonism assay		
Reference ligand	5α-Dihydrotestosterone	n.a.
Final concentration of reference ligand	1 μM	n.a.
Antagonism assay		
Reference ligand	5α-Dihydrotestosterone	5α-Dihydrotestosterone
Final concentration of reference ligand	n.p.	n.p.

Abbreviations: n.a. = not applicable;
n.p. = not provided

AR TA Assays Using Human Cells

Reference	Wilson et al. (2002)	Hartig et al. (2002)
Characteristics of Cell Line		
Name of cell line	MDA-kb2	MDA-MB-453
Cell source	Derived from MDA-MB-453 cells (human breast carcinoma)	Human breast carcinoma
Transfection of Cells with Plasmids		
Stable or transient tranfection	Stable reporter	Transduced reporter
AR expression vector	Endogenous hAR	Endogenous hAR
AR source	human	human
Reporter vector	pMMTV.Luc.neo	Ad/mLuc7
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	none	none
Endpoint measured for cell toxicity	n.a.	n.a.
Preparation of Cells for Assay		
Transient		
Pregrowth of cells before transient transfection	n.a.	24 hours
Time from transient transfection to treatment of cells	n.a.	4 hours
Stable		
Plating time prior to treatment with test substance	4 to 6 hours	n.a.
Transcriptional Activation Assay		
Test substance solvent	Ethanol	Ethanol
No. of replicates	At least 4	3 to 4
No. of times assay repeated	3	n.p.
Test substance incubation time	overnight	48 hours
Agonism assay		
Reference ligand	n.a.	5α-Dihydrotestosterone
Final concentration of reference ligand	n.a.	0.1 nM
Antagonism assay		
Reference ligand	5α-Dihydrotestosterone	5α-Dihydrotestosterone
Final concentration of reference ligand	0.1 or 1.0 nM	0.1 nM

Abbreviations: n.a. = not applicable;
n.p. = not provided

AR TA Assays Using Human Cells

Reference	Lambright et al. (2000)	Sultan et al. (2001)
Characteristics of Cell Line		
Name of cell line	MDA-MB-453-kb2	PALM
Cell source	Human breast carcinoma	Derived from PC-3 cells (human prostate adenocarcinoma)
Transfection of Cells with Plasmids		
Stable or transient tranfection	Stable reporter	Stable AR/Stable reporter
AR expression vector	Endogenous hAR	pSG5-puro-hAR
AR source	human	human
Reporter vector	MMTV.neo.luciferase	pMMTV-neo-Luc
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	pCMVβ-gal	none
Endpoint measured for cell toxicity	β-galactosidase activity	n.a.
Preparation of Cells for Assay		
Transient		
Pregrowth of cells before transient transfection	n.a.	n.a.
Time from transient transfection to treatment of cells	n.a.	n.a.
Stable		
Plating time prior to treatment with test substance	5 to 6 hours	n.p.
Transcriptional Activation Assay		
Test substance solvent	Ethanol	n.p.
No. of replicates	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.
Test substance incubation time	overnight	n.p.
Agonism assay		
Reference ligand	n.a.	n.a.
Final concentration of reference ligand	n.a.	n.a.
Antagonism assay		
Reference ligand	5α-Dihydrotestosterone	Methyltrienolone
Final concentration of reference ligand	0.1 nM	0.1 nM

Abbreviations: n.a. = not applicable;
n.p. = not provided

AR TA Assays Using Human Cells

Reference	Terouanne et al. (2000)	Terouanne et al. (2000)
Characteristics of Cell Line		
Name of cell line	PALM	PC-3
Cell source	Derived from PC-3 cells (human prostate adenocarcinoma)	Human prostate adenocarcinoma
Transfection of Cells with Plasmids		
Stable or transient tranfection	Stable AR/Stable reporter	Transient AR/Transient reporter
AR expression vector	pSG_5-puro-hAR	pSG_5-puro-hAR
AR source	human	human
Reporter vector	pMMTV-neo-Luc	pMAMneo-Luc
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	none	$pCMV_5$-β-galactosidase
Endpoint measured for cell toxicity	n.a.	β-galactosidase activity
Preparation of Cells for Assay		
Transient		
Pregrowth of cells before transient transfection	n.a.	n.p.
Time from transient transfection to treatment of cells	n.a.	n.p.
Stable		
Plating time prior to treatment with test substance	12 hours	n.a.
Transcriptional Activation Assay		
Test substance solvent	F12 medium	n.p.
No. of replicates	2	2
No. of times assay repeated	At least 3	At least 3
Test substance incubation time	30 hours	n.p.
Agonism assay		
Reference ligand	Methyltrienolone	n.a.
Final concentration of reference ligand	0.1 nM	n.a.
Antagonism assay		
Reference ligand	Methyltrienolone	Methyltrienolone
Final concentration of reference ligand	0.1 nM	0.1 nM

Abbreviations: n.a. = not applicable;
n.p. = not provided

AR TA Assays Using Human Cells

Reference	Schrader and Cooke (2000)
Characteristics of Cell Line	
Name of cell line	PC-3 LUC^{AR+}
Cell source	Derived from PC-3 cells (human prostate adenocarcinoma)
Transfection of Cells with Plasmids	
Stable or transient tranfection	Stable AR/Stable reporter
AR expression vector	pCMV5-hAR
AR source	human
Reporter vector	MMTV.pMAMneo-Luc
Endpoint measured	Luciferase activity
Plasmid transfected for cell toxicity	none
Endpoint measured for cell toxicity	n.a.
Preparation of Cells for Assay	
Transient	
Pregrowth of cells before transient transfection	n.a.
Time from transient transfection to treatment of cells	n.a.
Stable	
Plating time prior to treatment with test substance	24 hours
Transcriptional Activation Assay	
Test substance solvent	DMSO
No. of replicates	n.p.
No. of times assay repeated	n.p.
Test substance incubation time	18 hours
Agonism assay	
Reference ligand	5α-Dihydrotestosterone
Final concentration of reference ligand	n.p.
Antagonism assay	
Reference ligand	5α-Dihydrotestosterone
Final concentration of reference ligand	n.p.

Abbreviations: n.a. = not applicable;
n.p. = not provided